Reply to Office Action dated 25 April 2007

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph at page 15, lines 11-17 of the specification with the

following amended paragraph:

Examples of flocculants that can be used include, without limitation synthetic flocculants.

such as polyacrylamides, quaternary acrylate salts and natural flocculant macromolecules such as  $\frac{1}{2}$ 

chitosan, a natural polymer derived from chitin. Particular examples of flocculants include

polyacrylamide (PAM) flocculants such as Tramfloe TRAMFLOC® (Tramfloc Inc.), the cationic flocculant SURFLOC® 34030 (Jes-Chem Ltd.), polyacrylamide (PAM) flocculants such

as an Aquamark® AQUAMARK® AQ 600 Series flocculant, or a SuperFloe® SUPERFLOC® C-

500 Series flocculant (OEMI Inc.).

following amended paragraph:

In the second step, any starch or related material that is present may be digested using an

Please replace the paragraph at page 16, lines 15-29 of the specification with the

enzyme, such as, but not limited to an amylase. The enzyme may be used at a concentration of

from about 0.05% to about 0.20% (vol/vol), from about 0.09% to about 0.15% (vol/vol), or from

about 0.09% to about 0.11% (vol/vol). If an amylase is used, it is preferred that the alkaline

solution be brought to an approximately neutral value of pH (i.e. ~pH 7) before adding the amylase. In an example, the solution containing the amylase is heated to a temperature of from

about 50°C to about 100°C, or from about 70°C to about 90°C for about 20 to about 30 minutes

to gelatinize the starch. The amylase will hydrolyse the starch and any related material.

Generally, the amylase that is chosen to break down the starch material should be functional and stable within the temperature ranges indicated above. It is particularly preferred that the amylase

not require a calcium co-factor to digest the starch material. Examples of such an amylase.

include, without limitation, Termamyt<sup>®</sup> TERMAMYL<sup>®</sup> LC (Novozymes A/S), an α-amylase

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enzyme for starch liquefaction at low calcium levels, and Spezyme\* SPEZYME\* FRED

(Genencor International Inc.), a starch hydrolyzing  $\alpha$ -amylase with high heat and low pH

stability.

Please replace the paragraph at page 17, lines 7-14 of the specification with the following

amended paragraph:

The resulting acidified solution can then be filtered to remove any particulates and

microbiological contaminants, through a filter pad that preferably has a cutoff point of about 20

 $\mu m.$  This filter may be coated with a pre-coat of a filter aid having a thickness of about 2 to

about 5 mm, such as Celite® CELPURE® C65 (diatomaceous earth having a permeability of

0.065 Darcy; World Minerals), which has a nominal porosity of about 0.2 μm. An equivalent

weight of a filter-aid, for example, an acid-washed pharmaceutical grade filter-aid, such as Celite\* CELPURE\* C300 (diatomaceous earth having a permeability of 0.300 Darcy; World

Minerals), may also be added as a body feed to the acidified solution prior to conducting the

filtration step.

Please replace the paragraph at page 18, lines 27-31 of the description with the following

amended paragraph:

To prevent gellation of the cereal  $\beta$ -glucan at each of the steps of the purification method

of the present invention, it is preferred that the addition of salts be minimized throughout the

process. For example, it is preferred that reverse osmosis (RO) purified or deionized (DI) water be used, as well as an amylase not requiring a calcium cofactor, such as <del>Termamylase</del>

TERMAMYL® LC (Novozymes A/S).

Please replace the text at page 25, line 27 to page 26, line 22 of the specification with the

following amended text:

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Oat bran (The Ouaker Oats Company) was slurried with alkaline reverse osmosis (RO) water at a pH of about 9.5 to a final solids concentration of 4-10%. The temperature was maintained at 45°C ± 5°C. The cereal β-glucan was extracted from the oat bran over a period of 30 minutes. After this time, the solids were removed by centrifugation with a decanter centrifuge. The centrate was cooled to room temperature, and the cationic flocculant SURFLOC® 34030 (Jes-Chem Ltd.) was added at a 0.2% concentration. Following an incubation period of 20 minutes, coagulated particulate material was removed by centrifugation using a disk-stack centrifuge. The pH of the centrate was adjusted to approximately neutral, heated to >72°C to gelatinize starch, and treated with the heat-stable amylase Termamy! TERMAMYL. LC (Novozymes A/S). When the solution no longer produced a positive jodine test, the pH was reduced to about 4.0 to inactivate the enzyme, and the mixture was heated to 85°C for 30 minutes to denature the protein present. The solution was cooled to 4°C for one hour, and then heated to a temperature of about 72°C. An equivalent weight of CELITE® CELPURE® C300 (diatomaceous earth having a permeability of 0.300 Darcy; World Minerals) was added to the solution, and the mixture was then filtered using a filter-press containing 25 µm filter-papers and pre-coated to a depth of about 4 mm with CELITE® CELPURE® C65 (diatomaceous earth having a permeability of 0.065 Darcy; World Minerals). The filter press was preheated to a temperature of about 65°C, and the pH of the feedstream for the filter press was adjusted to 4.5 before the  $\beta$ -glucan solution was filtered. After the  $\beta$ -glucan solution was passed through the filter, the press was flushed with reverse osmosis water resulting in a clear, pale vellow coloured β-glucan solution. The β-glucan solution was cooled to 5°C and 95% ethanol at a temperature of -20°C was added to a final volume of about 15% (w/w) with stirring. A suspension of β-glucan was formed that was immediately separated from the solution by centrifugation with a disk-stack centrifuge. The isolated solid β-glucan was added to RO water at 45°C, allowed to disperse and then heated to between 60-70°C to produce a clear colorless solution containing about 1% βApplication Serial No. 10/554,288 Amendment dated 25 October 2007 Reply to Office Action dated 25 April 2007

glucan. The separated β-glucan was colourless, had a purity of greater than 75%, a viscosity >500 cP, and an exception clarity <50 NTU, as measured using a turbidity meter.

Please replace the text at page 27, line 21 to page 28, line 4 of the specification with the following amended text:

At the end of the incubation period, swab samples of the skin sections were taken with both dry cotton gauze swabs and cotton gauze swabs moistened with 0.2 mL of 70% methanol/H<sub>2</sub>O. The skin sections were removed from the Phaeoeell® PHACOCELL® chamber and immediately frozen in liquid nitrogen. The skin sections were then cut into 15 μm slices from the horny layer to the deeper dermis. The skin sections were allowed to air dry on clean glass slides and not fixed with any fluid. The slices were then stained with BACTIDROP<sup>TM</sup> Calcofluor White for 30 seconds and then washed of excess stain with deionized water. The staining and washing steps were repeated twice. The stained sample was covered with a clean glass cover slip and examined by fluorescence with a LEIKA® fluorescent microscope having an exciter filter ranging between 400-500 nm with a peak of 440 nm, a barrier filter of 500-520 nm, and a xenon are (burner) lamp. BACTIDROP<sup>TM</sup> Calcofluor White is a non-specific fluorochrome that binds to cellulose, and upon excitation with long wavelength ultraviolet light delineates the cell walls of cellulose-containing organisms. The deposition of the β-glucan molecules was monitored and quantified using bright fluorescence, focus inverted to white spots (3 – 5 μm) seen upon the cell walls of the samples and in the intercellular interstices.